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Appearance and Chemical Composition of Eastern Cottonwood Grown Under Nutrient Deficient Conditions

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Appearance and Chemical Composition of Eastern Cottonwood Grown Under Nutrient Deficient Conditions

JOHN HACSKAYLO and JOHN P. VIMMERSTEDT[1]

INTRODUCTION

Eastern cottonwood (*Populus deltoides* Bartr.) is one of the best sources of fiber for high quality printing papers. With increasing demand for this type of wood fiber, thousands of acres of cottonwood are planted annually.

Eastern cottonwood ranks among the fastest growing tree species in North America. However, little is known of its inorganic nutrient element requirements and the effects of deficiencies on its visual and chemical characteristics. This paper reports the visual and chemical effects which occurred when specific essential nutrients were omitted from cultures of cottonwood cuttings grown in the greenhouse. The anatomical changes resulting from the nutrient deficiencies are described in another paper (2).

MATERIALS AND METHODS

Cuttings from a Wisconsin clone were rooted in quartz sand and watered daily until the plants were about 10 cm. tall. The rooted cuttings were then removed from the flats, rinsed twice in deionized water (xxH_2O), and planted in 3-gallon, polyethylene-lined crocks. The crocks were filled with HCl-washed silica quartz and were equipped for continuous aeration (Fig. 1).

Light intensity in the greenhouse varied from 300 to 12,000 foot candles and photoperiod was 15 hours per day. At any given time, light intensity was reasonably uniform over the entire group of plants. Air temperatures ranged from 70° to 100° F. during the day and from 60° to 80° F at night. Night temperatures were normally 20° below day temperatures.

Two cuttings were placed in each of 45 crocks, which were divided into three groups of 15 crocks. A group consisted of one crock each of

[1]Dr. John Hacskaylo, professor of forestry at the Ohio Agricultural Research and Development Center, died September 11, 1965. Dr. John P. Vimmerstedt is assistant professor of forestry, Ohio Agricultural Research and Development Center.

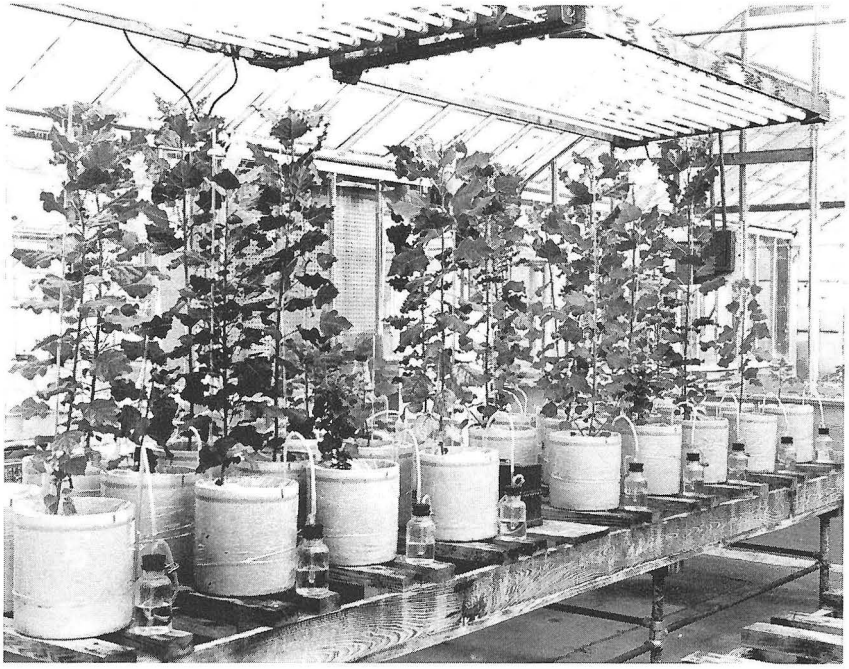

Fig. 1.—Greenhouse arrangement of cottonwood nutrition experiment.

all nutrient-deficient solutions, two of the complete solution, and one of deionized water. The composition of the nutrient solutions is given in Table 1.

Nutrient solutions were prepared from reagent grade chemicals and deionized water. Solution pH was initially adjusted to 5.4 with 0.1% NaOH. The nutrient solutions were replaced every 2 weeks. Deficient nutrient solutions are referred to as —N, —P, —K, —Ca, —Mg, —S, —Fe, —Mn, —Zn, —B, —Cu, —Mo, and xxH_2O.

Plant diameter was measured level with the top of the crock. Height measurements were also made from this reference point.

Thirty plants, one from each crock in two replicates, were harvested 44 days after transplanting. Another 30 plants were removed for anatomical study 65 days after transplanting. The remaining 30 plants, one from each crock in two replicates, were harvested after 77 days for chemical analyses.

The plants were divided into roots, stems, and leaves and were dried to a constant weight at 80° C. The dried plant parts were ground to

TABLE 1.—Composition of Nutrient Solutions.

Compounds	Comp.	—N	—P	—K	—Ca	—Mg	—S	—Fe	—B	—Zn	—Cu	—Mn	—Mo
	Number of Millimoles of Compound Added per Liter of Nutrient Solution												
KNO_3	2		2		2	2	2	2	2	2	2	2	2
KH_2PO_4	2	2			2	2	2	2	2	2	2	2	2
$Ca(NO_3)_2 \cdot 4H_2O$	3		3	3		3	3	3	3	3	3	3	3
$MgSO_4 \cdot 7H_2O$	2	2	2	2				2	2	2	2	2	2
KCl		2	2										
$CaCl_2$		3											
Na_2SO_4					2	2							
NaH_2PO_4				2									
$NaNO_3$				2									
$Mg(NO_3)_2$					3								
$MgCl_2$							2						
	Number of Micromoles per Liter of Nutrient Solution												
Fe-EDTA	89	89	89	89	89	89	89	0	89	89	89	89	89
H_3BO_3	37	37	37	37	37	37	37	37	0	37	37	37	37
$MnCl_2 \cdot 4H_2O$	7	7	7	7	7	7	7	7	7	7	7	0	7
$ZnCl_2$	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0	0.76	0.76	0.76
$CuCl_2 \cdot 2H_2O$	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0	0.31	0.31
MoO_3	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0

pass a 60 mesh screen. N content was determined by Kjeldahl digestion (6) and S by the method of Butters and Chenery (1). P, K, Ca, Mg, Fe, Mn, Zn, B, Cu, and Mo contents were determined by spectrographic analysis of a sample ashed in a muffle furnace (7).

Duncan's Multiple Range Test, as modified by Kramer (5) for unequal numbers of replications, was used to test for significant differences between plants grown in the complete nutrient solution and those grown in deficient solutions. Characteristics tested were height and diameter growth, dry weight, and top to root ratio.

RESULTS

Cuttings grown in the complete nutrient solution had large green leaves, rapid height and diameter growth, and white, many-branched root systems. Figures 2 and 3 illustrate differences in appearance between plants in the complete solution and those in the nutrient deficient solutions. Figures 4, 5, and 6 summarize the height growth, diameter growth, and dry weight measurements, respectively. Table 2 lists average top to root ratios and Tables 3a, 3b, and 3c give average concentrations of nutrient elements in leaves, stems, and roots. The effects of each deficiency are described below.

Nitrogen

The initial response to nitrogen deficiency was yellowing of the lower leaves. Later all leaves became pale yellow and newly expanded leaves were small. The root systems looked like those in the complete solution.

After 77 days, height growth, diameter growth, dry weight, and top to root ratios were all significantly lower in the —N plants than in those in the complete nutrient solution. The concentration of N in the plant tissue was much lower in the —N treatment than in plants in the complete solution. Values were:

	Leaves	Stems	Roots
—N (N, %)	1.38	0.41	0.50
Complete (N, %)	2.86	1.01	1.27

Phosphorus

With time, the veins of the upper leaves turned pink but the lower leaves maintained a healthy appearance. The size and complexity of the root system were reduced.

After 77 days, height and diameter growth, dry weight, and top to root ratios were significantly lower in the —P plants than in plants in the complete solution. Plants grown in the —P solution had much lower concentrations of P in their tissue than those grown in the complete solution. Values were:

Fig. 2a.—Appearance of cottonwood cuttings grown for 77 days in complete, —N, —P, —K, and xxH_2O nutrient solutions.

	Leaves	Stems	Roots
—P (P, %)	0.14	0.05	0.10
Complete (P, %)	0.83	0.43	0.91

Potassium

The lower leaves developed a distinctive pattern of chlorosis and browning between the veins, while the upper leaves appeared normal. The root systems were sparse and darker colored than those in the complete solution.

After 77 days in the —K solution, height and diameter growth and dry weight were significantly less than in plants in the complete solution. K concentrations were markedly lower in the —K treatment. Values were:

	Leaves	Stems	Roots
—K (K, %)	0.44	0.20	0.22
Complete (K, %)	4.59	1.46	2.06

Calcium

Basal leaves were dark green but those near the tip were small, yellow, and eventually died. The growing tip also died. The root system was quite dark and very small.

Fig. 2b.—Appearance of cottonwood cuttings grown for 77 days in complete, —Ca, —Mg, —S, and xxH_2O nutrient solutions.

After 77 days, height and diameter growth and dry weight were lower than that of plants in the complete nutrient solution. Top to root ratios were higher. Plants grown in the —Ca solutions had much lower concentrations of Ca than those grown in the complete solution. Values were:

	Leaves	Stems	Roots
—Ca (Ca, %)	0.20	0.15	0.15
Complete (Ca, %)	0.95	0.46	0.83

Magnesium

Leaves on the lower and middle parts of the stems of cottonwood grown in —Mg solutions developed yellow-green chlorotic areas between the veins. However, the leaves near the tip appeared normal. Roots were light colored and sparse.

Mg-deficient plants did not differ from those in the complete solution in height or top to root ratio but were significantly smaller in diameter growth and dry weight. The —Mg treatment markedly lowered the Mg concentration in the plants. Values were:

	Leaves	Stems	Roots
—Mg (Mg, %)	<0.04	<0.04	<0.04
Complete (Mg, %)	0.49	0.17	0.24

Fig. 2c.—Appearance of cottonwood cuttings grown for 77 days in complete, —Fe, —Mn, —Zn, and xxH_2O nutrient solutions.

Sulfur

Above-ground symptoms resembled nitrogen deficiency. Leaves were small and yellow-green. Root systems were poorly developed and greyish brown.

After 77 days, height and diameter growth, dry weight, and top to root ratios were all significantly lower than in plants in the complete nutrient solution. Comparative S concentrations in the S-deficient plants and those grown in the complete solution were:

	Leaves	Stems	Roots
—S (S, %)	0.13	0.04	0.04
Complete (S, %)	0.38	0.05	0.18

Iron

The pale yellow, chlorotic appearance of the leaves near the tip contrasted sharply with the green leaves below. Root systems of the Fe-deficient trees were very light brown and fairly well developed.

Fe-deficient plants did not differ from those in the complete solution in height or diameter growth, dry weight, or top to root ratio. Concentrations of Fe in the Fe-deficient plants were well below those in the complete solution. Values were:

Fig. 2d.—Appearance of cottonwood cuttings grown for 77 days in complete, —B, —Cu, —Mo, and xxH_2O nutrient solutions.

	Leaves	Stems	Roots
—Fe (Fe,ppm)	33	11	74
Complete (Fe,ppm)	90	24	925

Manganese

The upper leaves developed grey mottling. Roots were white but rather sparse. After 77 days, diameter growth and dry weight were significantly lower than in plants grown in the complete nutrient solution. Height growth and top to root ratio of Mn-deficient plants did not differ significantly from those in the complete solution. Concentrations of Mn in the leaves and roots of the Mn-deficient plants were well below those in the complete solution. Stem concentrations were not greatly different. Values were:

	Leaves	Stems	Roots
—Mn (Mn,ppm)	<9	<9	<9
Complete (Mn,ppm)	49	9	37

Zinc and Molybdenum

Deficiency symptoms for these elements were not induced. The —Zn and —Mo treatments resulted in plants which did not differ significantly from those in complete solution in dry weight, height

TABLE 2.—Average Top to Root Ratios for Each Treatment After 44 and 77 Days' Growth.

Treatment	Shoot to Root Ratio at 44 Days Mean	Shoot to Root Ratio at 77 Days Mean
Complete	9.05	6.09
—N	9.21	2.42*
—P	10.90	3.70*
—K	9.14	7.22
—Ca	9.06	13.88*
—Mg	12.71*	9.74*
—S	3.10*	3.62*
—Fe	10.75	4.62
—Mn	10.53	6.75
—B	5.37*	4.90
—Zn	11.95*	4.75
—Cu	8.94	6.31
—Mo	12.44*	4.47

*Differs significantly from the complete.

growth, or diameter growth. The concentration of Zn in the —Zn tissue and the concentration of Mo in the —Mo tissue was actually higher than in plants in the complete solution.

Boron

The most noticeable symptom of B deficiency was a marked reduction in height growth and size of the terminal leaves. Leaf color was similar to that of plants in the complete solution.

Height and diameter growth and dry weight were significantly lower in the B-deficient plants than those in the complete solution. The root systems were well developed. B concentrations were lower in the B-deficient plants than in plants in the complete solution and were markedly lower in the case of the leaves. Actual concentrations were:

	Leaves	Stems	Roots
—B (B,ppm)	9	7	10
Complete (B,ppm)	68	13	15

Copper

The lower leaves were dark green with grey and light brown mottling. Upper leaves were small.

Height growth was significantly less than in the complete solution but diameter growth, dry weight, and top to root ratio were not. Root systems were well developed. Cu concentrations were lower in the leaves and roots of the plants grown in the —Cu nutrient solution than

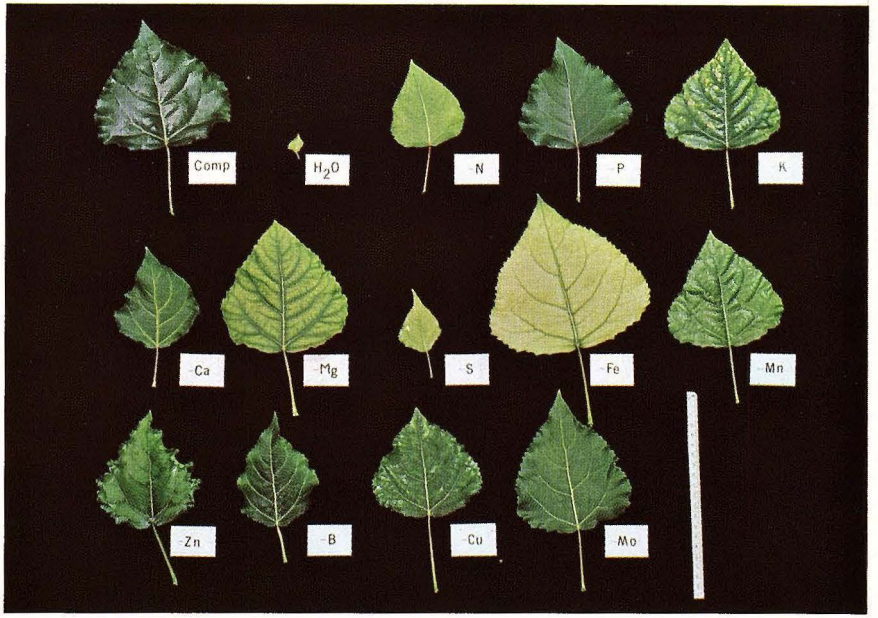

Fig. 3.—Typical leaves of cottonwood grown in nutrient solutions (above) and typical root systems of cottonwood grown in nutrient solutions (below). Top row: complete, xxH_2O, —N, —P, —K; middle row: —Ca, —Mg, —S, —Fe, —Mn; bottom row: —Zn, —B, —Cu, —Mo.

in those grown in the complete solution. For the stems, the reverse was true. Values were:

	Leaves	Stems	Roots
—Cu (Cu,ppm)	trace	2.8	<2.5
Complete (Cu,ppm)	2.2	0.6	8.0

DISCUSSION

Several of the responses of cottonwood to nutrient deficiencies noted above were similar to those observed previously (3) in sweetgum (*Liquidambar styraciflua* L.) and black locust (*Robinia pseudoacacia* L.). For example, in those species, Fe-deficient terminal leaves were chlorotic between the veins, K deficiency produced browning of the lower leaves, Ca and S deficiency led to yellowing and dieback of the terminal parts, and N deficiency produced chlorosis and stunting.

Experiments of this type do not explain the underlying physiological processes responsible for the development of deficiency symptoms. Keller

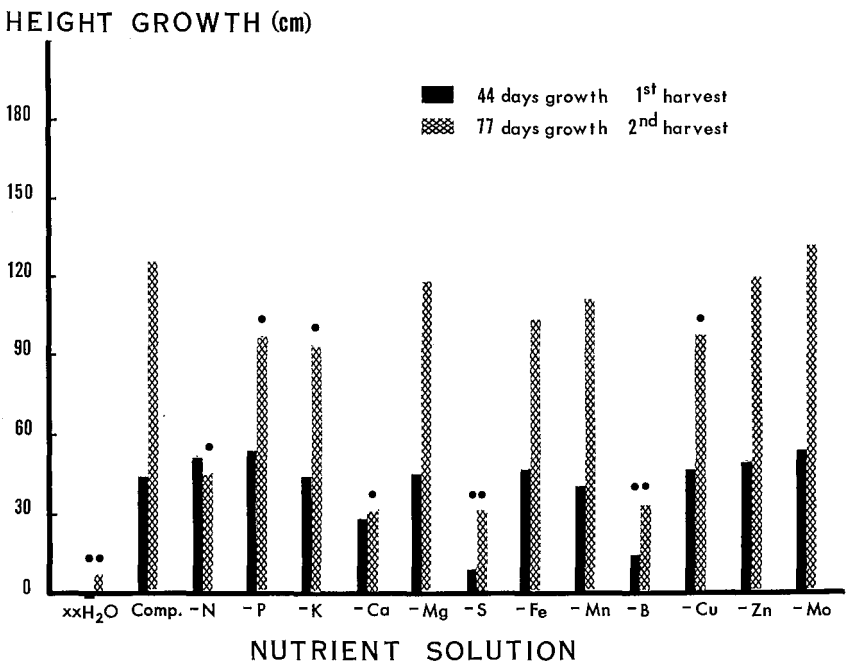

Fig. 4.—Average height growth of eastern cottonwood cuttings after 44 and 77 days. Each bar is the average of two trees. Dot indicates statistically significant difference from complete nutrient solution.

and Koch (4) studied the effect of N and Fe nutrition on CO_2 exchange and chlorophyll content in *Populus euramericana marilandica.* N-deficient leaves (1.80% N) and "well fed" leaves (3.40% N) had the same net assimilation of CO_2 in weak light. In strong light, however, net assimilation of the nitrogen-deficient leaves per unit area was only 60% of that of the normal leaves. Chlorophyll content per unit of leaf area was positively correlated with N content.

They also found 50-60 ppm of Fe to be the upper limit of latent iron deficiency. There was a good correlation between Fe and chlorophyll contents and between Fe content and CO_2 uptake.

These results provide a physiological explanation for responses to N and Fe deficiencies; i.e., N and Fe deficiencies lead to reduced chlorophyll contents, resulting in lowered photosynthate production and reduced growth.

The rooted cottonwood cuttings contained some nutrients when they were transplanted into the deficient solutions. The rooting medium, reagents, and dust probably were sources of contamination during the

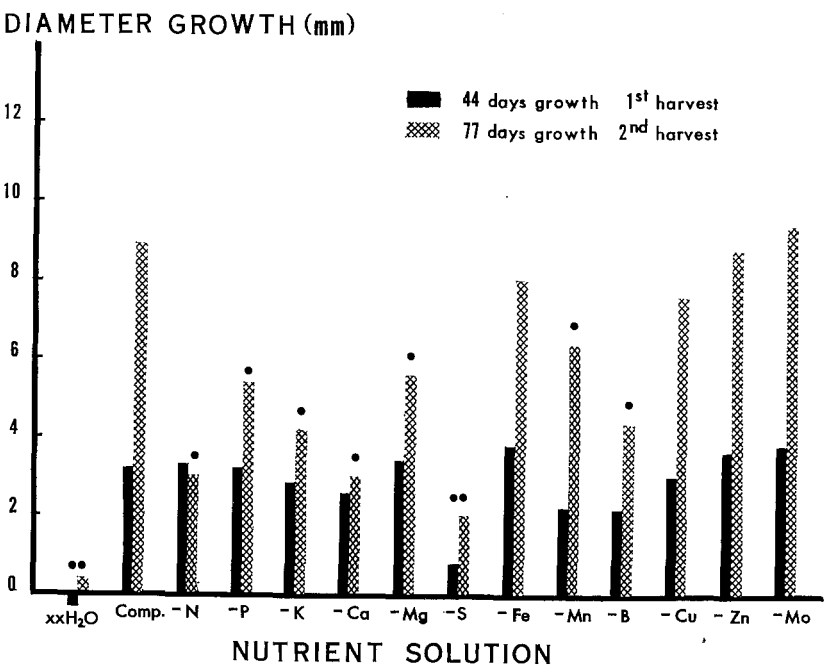

Fig. 5.—Average diameter growth of eastern cottonwood cuttings after 44 and 77 days. Each bar is the average of two trees. Dot indicates statistically significant difference from complete nutrient solution.

experiment. Thus, the growth responses observed represent a balance between inherent sensitivity to low levels of essential nutrients and the extent to which these elements were excluded from the growth medium. More rigorous control of contamination, such as use of recrystallized reagents, glass distilled water, and solution culture instead of sand culture, might have resulted in development of more pronounced growth differences and deficiency symptoms earlier in the experiment.

For elements other than Zn and Mo, this report may be helpful in diagnosing possible nutrient element deficiencies of cottonwood, especially in young plants. Where visual symptoms suggest a deficiency, the values obtained in the tissue analyses (Table 3) could be used to confirm or identify the deficient element. If facilities for tissue analyses are lacking, a useful alternative is to paint individual leaves showing deficiency symptoms with dilute solutions of the various essential elements. Leaves painted with the deficient element will often look better in 1 or 2 weeks. After the deficient element is identified, a fertilization program can be planned to correct the deficiency.

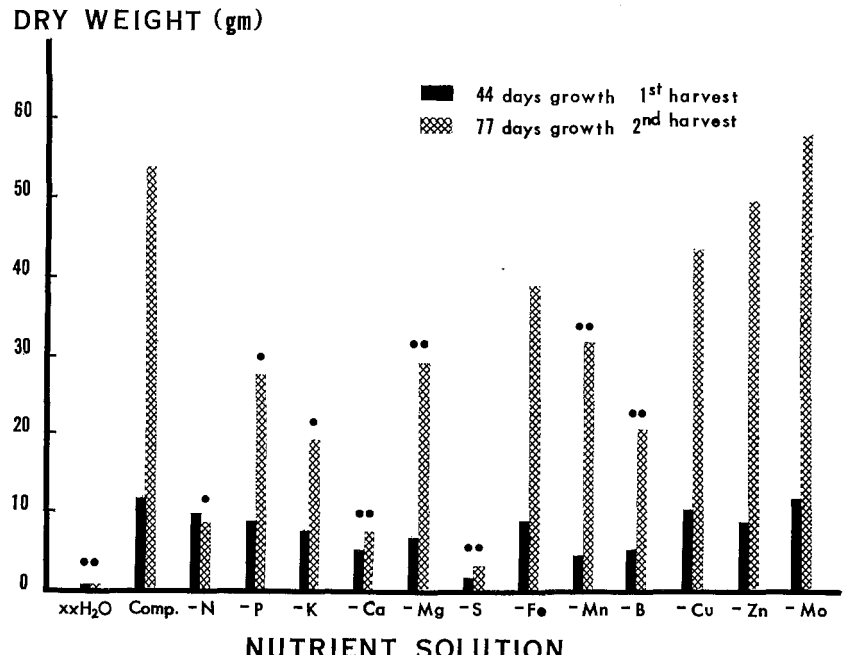

Fig. 6.—Average dry weight of eastern cottonwood cuttings after 44 and 77 days' growth. Each bar is the average of two trees. Dot indicates statistically significant difference from complete nutrient solution.

TABLE 3a.—Concentration of Nutrient Elements in Leaves of Cottonwood After 77 Days' Growth in Various Nutrient Solutions.

Nutrient Solution	N	P	K	Ca	Mg	S	Fe	Mn	Zn	B	Cu	Mo
	Percent of Oven-Dry Weight						Parts per Million					
Complete	2.86	0.83	4.59	0.95	0.49	0.378	90	49	14	68	2.2	1.3
—N	**1.38**	1.47	4.16	1.61	0.37	0.306	79	69	64	66	10.4	1.1
—P	2.74	**0.14**	3.86	0.96	0.30	0.331	108	63	61	64	6.0	0.9
—K	2.56	1.48	**0.44**	1.61	0.99	0.490	45	82	35	82	16.1	8.2
—Ca	2.01	0.71	2.85	**0.20**	0.53	0.294	53	36	27	31	<2.7	1.2
—Mg	2.74	1.02	6.54	1.08	**<0.04**	0.497	79	57	30	62	<2.6	0.4
—S	2.80	1.97	5.87	1.46	0.46	**0.128**	100	167	74	85	Tr.*	>9.0
—Fe	3.34	1.06	4.60	1.17	0.60	0.450	**33**	127	36	79	<4.9	2.2
—Mn	4.17	0.93	4.80	0.98	0.58	0.469	95	**<9**	36	78	2.0	1.8
—Zn	3.04	0.86	4.96	1.22	0.64	0.547	87	85	**36**	98	4.4	3.7
—B	2.61	0.77	4.33	1.12	0.27	0.438	65	96	48	**9**	4.0	1.2
—Cu	3.55	0.84	4.41	1.12	0.47	0.481	97	47	23	62	**Tr.***	1.4
—Mo	3.14	0.93	5.10	1.15	0.67	0.503	95	46	29	>85	<2.5	**1.8**

*Tr. = Trace

TABLE 3b.—Concentration of Nutrient Elements in Stems of Cottonwood After 77 Days' Growth in Various Nutrient Solutions.

Nutrient Solution	N	P	K	Ca	Mg	S	Fe	Mn	Zn	B	Cu	Mo
	Percent of Oven-Dry Weight						Parts per Million					
Complete	1.01	0.43	1.46	0.46	0.17	0.051	24	9	8	13	0.6	0.3
—N	**0.41**	0.56	1.68	0.81	0.14	0.012	27	18	44	18	1.7	0.3
—P	0.97	**0.05**	1.72	0.72	0.16	0.106	23	19	57	17	<3.1	0.3
—K	0.85	0.47	**0.20**	0.67	0.16	0.050	17	16	37	16	4.0	0.4
—Ca	1.10	0.72	1.62	**0.15**	0.47	0.137	19	18	27	18	3.7	0.7
—Mg	0.80	0.60	1.49	1.18	<**0.04**	0.090	16	17	20	16	2.5	0.2
—S	1.60	1.04	1.35	0.81	0.20	**0.038**	18	48	36	16	Tr.	>9.0
—Fe	1.03	0.53	1.71	0.59	0.22	0.096	**11**	30	22	18	3.4	0.5
—Mn	1.12	0.49	1.25	0.61	0.25	0.053	18	<**9**	18	17	2.8	0.2
—Zn	1.37	0.47	1.54	0.53	0.24	0.168	28	22	**26**	16	4.0	0.7
—B	2.34	0.75	2.50	0.96	0.21	0.172	32	33	34	**7**	3.5	0.5
—Cu	1.37	0.54	2.04	0.56	0.24	0.078	25	13	16	15	**2.8**	0.4
—Mo	1.28	0.57	1.86	0.54	0.26	0.084	37	14	21	19	3.4	**0.7**

TABLE 3c.—Concentration of Nutrient Elements in Roots of Cottonwood After 77 Days' Growth in Various Nutrient Solutions.

Nutrient Solution	N	P	K	Ca	Mg	S	Fe	Mn	Zn	B	Cu	Mo
	Percent of Oven-Dry Weight						Parts per Million					
Complete	1.27	0.91	2.06	0.83	0.24	0.178	925	37	13	15	8.0	0.7
—N	**0.50**	0.93	2.12	0.70	0.13	0.100	1032	42	66	37	12.9	1.4
—P	0.81	**0.10**	1.69	0.75	0.14	0.188	1374	74	33	37	11.7	0.4
—K	1.25	1.30	**0.22**	1.27	0.33	0.163	1050	200	32	22	5.3	2.8
—Ca	1.00	0.63	0.74	**0.15**	0.53	0.203	1300	37	29	21	12.5	1.8
—Mg	0.98	1.39	0.86	1.92	<**0.04**	0.172	884	188	29	17	13.0	0.5
—S	1.27	1.29	1.42	0.86	0.18	**0.041**	1440	182	47	18	8.1	>9.0
—Fe	1.31	1.03	2.12	0.85	0.24	0.188	**74**	108	36	18	30.0	1.6
—Mn	1.35	1.21	1.66	0.95	0.22	0.131	534	<**9**	18	16	3.0	0.5
—Zn	1.31	0.90	2.21	0.78	0.27	0.090	588	44	**42**	16	6.6	1.2
—B	1.94	1.23	2.66	1.25	0.18	0.197	728	204	36	**10**	12.0	1.2
—Cu	1.53	1.05	2.18	0.69	0.23	0.131	656	44	18	15	<**2.5**	0.5
—Mo	1.65	1.24	2.91	1.08	0.38	0.244	1250	50	32	23	5.0	**1.2**

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Muck Crops Branch, Willard, Huron County: 15 acres

North Central Branch, Vickery, Erie County: 335 acres

Northwestern Branch, Hoytville, Wood County: 247 acres

Southeastern Branch, Carpenter, Meigs County: 330 acres

Southern Branch, Ripley, Brown County: 275 acres

Vegetable Crops Branch, Marietta, Washington County: 20 acres

Western Branch, South Charleston, Clark County: 428 acres